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Original Article

First confirmation of the distribution of rice weevil, *Sitophilus oryzae*, in South Korea

Ki-Jeong Hong [a,*], Wonhoon Lee [b], Young-Ju Park [c], Jeong-Oh Yang [c]

[a] Department of Plant Medicine, Sunchon National University, Suncheon, 57922, Republic of Korea
[b] Department of Plant Medicine and Institute of Agriculture & Life Science, Gyeongsang National University, Jinju 52828, Republic of Korea
[c] Plant Quarantine Technology Research & Development, Animal and Plant Quarantine Agency, Gimcheon, 39660, Republic of Korea

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ABSTRACT

In Korea, the classification of the *Sitophilus* weevil group occurring in stored grains has been confused, resulting in its misidentification in most references reporting *Sitophilus oryzae* (Linnaeus). However, we recently found and identified rice weevil (*S. oryzae*) populations in stored rice grains in the rice processing complex in South Korea. Here, we report the distribution of rice weevil in South Korea and summarize morphological and molecular characteristics, as well as provide a pictorial identification key for *Sitophilus* weevils occurring in stored grains.

Introduction

Sitophilus weevils, including the rice weevil (*S. oryzae* (Linnaeus)), maize weevil (*S. zeamais* Motschulsky), and granary weevil (*S. granarius* (Linnaeus)), are well-known insect pests of stored grains worldwide. These weevils have a nearly cosmopolitan distribution, occurring throughout all warm and tropical parts of the world (CABI 2015). Generally, a female adult *Sitophilus* weevil bores a hole in a cereal grain, lays an egg, usually one egg per individual grain, and then seals the hole with a waxy secretion. Upon hatching, the larva develops while feeding inside the grain, then pupates. It usually leaves the grain completely hollow when it exits as an adult (Longstaff 1981).

The taxonomy of the *Sitophilus* group has been confused until now (CABI 2015). After first being described by Linnaeus in 1763 as *Curculio oryza*, many researchers subsequently recognized that two distinct forms of the species existed, which were described as “large” and “small” forms. In 1855, Motschulsky recognized the large form as a distinct species and named it *Sitophilus zeamais*. In 1928 and 1931, Takahashi complicated matters by raising the small form to a specific status as *Calandra sasakii*. Unfortunately, the size difference between *S. oryzae* and *S. zeamais* is not consistent, so it is not possible to be sure that references to the large and small forms of *Calandra oryzae* refer to *S. zeamais* and *S. oryzae*, respectively (CABI 2015; Morimoto 1961). Therefore, it is also possible that some references to *S. oryzae* published in the 1960s and early 1970s were actually referring to *S. zeamais* misidentified by use of old keys (CABI 2015). Currently, the genus *Sitophilus* and its species may be identified by using the keys of Gorham (1987).

In Korea, the classification of this group has also been confused. Currently, two species, *S. zeamais* and *S. oryzae*, are recorded in Korea; however, most references reporting *S. oryzae* have been dealing with the misidentified species, *S. zeamais* (Cho et al 1988; Hyun 1960; Hyun 1962; Hyun 1964; Hyun and Ryoo 1974; Kim and Ryoo 1982; Ryoo and Cho 1988; Ryoo et al 1988; Ryoo and Cho 1992; Yoo and Ryoo, 1989; Yoon et al 1997). Hong et al (2001) also confirmed specimens preserved in the Insect Collection of Seoul National University and reported that *S. oryzae* was not distributed in Korea.

We recently found and identified *S. oryzae* populations that were collected from stored rice grains in the rice processing complex (RPC) in Korea. Therefore, in this study, we report a distribution of rice weevil in South Korea and summarize morphological characteristics and molecular data for identification of *Sitophilus* weevils in stored grains in Korea.

* Corresponding author.
E-mail address: curcul@sunchon.ac.kr (K.-J. Hong).
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Materials and methods

Collecting specimens of Sitophilus weevils

We surveyed 52 RPCs throughout South Korea in 2015–2016 for stored rice insect pests using corrugated cardboard traps (30×30 cm) and by sampling rice grains. Two weevil pests, *S. zeamais* and *S. oryzae*, were mainly collected during this period. The specimens were deposited in the Insect Collection of Sunchon National University.

Morphological characteristics for identifying Sitophilus weevils

The genus *Sitophilus* contains four major economic pests, the granary weevil (*S. granarius*), tamarind weevil (*S. linearis*), rice weevil (*S. oryzae*), and maize weevil (*S. zeamais*). Adult samples

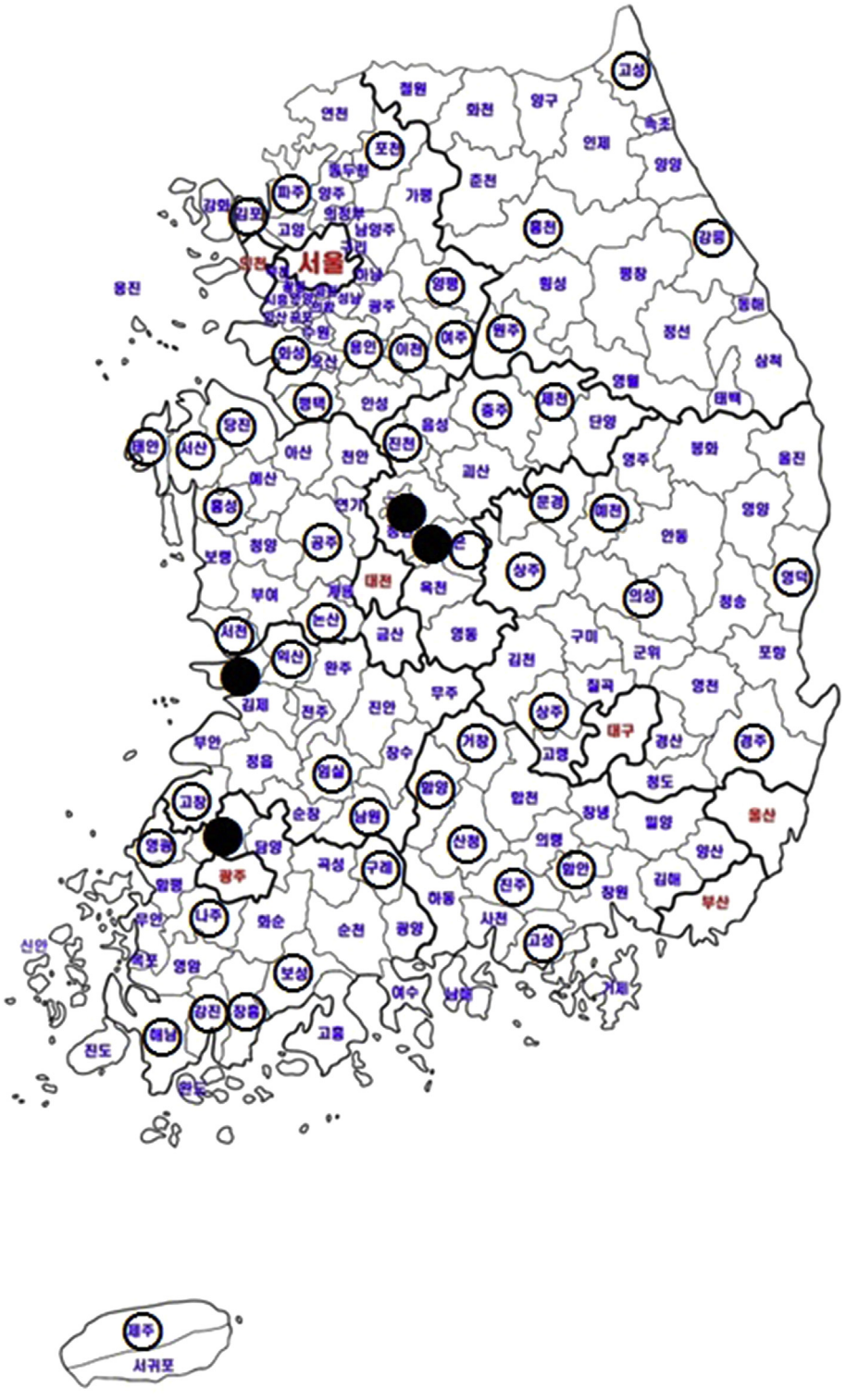

Figure 1. Populations of *Sitophilus zeamais* (○) and *S. oryzae* (●) were confirmed at rice processing complexes of South Korea in this survey (2015–2016).

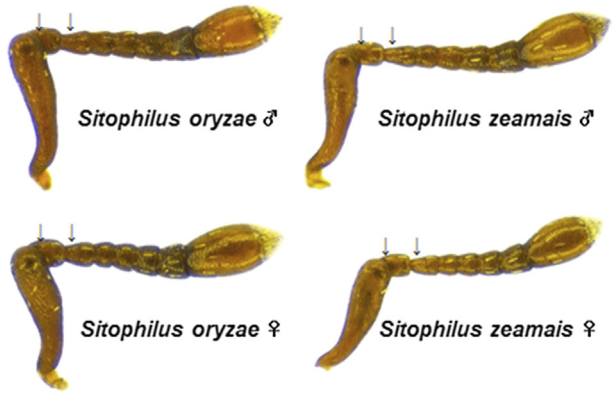

Figure 2. Comparisons of length of the antennal funicle I and II for identification of *Sitophilus zeamais* and *S. oryzae* described by Egorov and Zherikhin (1996). However, this characteristic is not entirely reliable for their identification.

were identified using the following external morphological characteristics: antennal scrobe, microsculptures of pronotum, length of antennal funicle I and II, and lateral elevation of scutellum. However, some of these external characters are not entirely reliable to distinguish adults of *S. oryzae* and *S. zeamais*. Therefore, we also used external genitalia characteristics for adults, as well as the epipharyngeal rod and apical sensory organ on the labial palp of larvae for identification of these two species. Many pictures of specimens displayed in these figures were taken using a Leica DFC2900 digital camera and stacked using the Leica Application Suite Program (LAS Version 4.6.0; http://www.leica-microsystems.com).

Additionally, an identification key for four *Sitophilus* species known as stored grain insect pests was also constructed based on morphological characteristics. These specimens were collected from Korea for the rice weevil (*S. oryzae*) and maize weevil (*S. zeamais*) and intercepted at the Korean borders for the granary weevil (*S. granarius*) and tamarind weevil (*S. linearis*).

Molecular identification of Sitophilus oryzae and S. zeamais

Genomic DNA was extracted from each specimen using a DNeasy Blood & Tissue kit (Qiagen, Inc., Dusseldorf, Germany) following the manufacturer's protocols. Two primer sets were used to amplify the COI gene, 5′-AGT TTG CTA ATT CGG GCA GA-3′ and 5′-ACT CCG GTT AAT CCT CCA AT-3′ for *S. oryzae* and 5′-GGG CTG AAT TAG GGA ATC CT-3′ and 5′-CTC CTG TTA ATC CCC CGA TA-3′ for *S. zeamais* (Corrêa et al 2013). The DNA fragments were amplified using master mix (GoTaq® Green Master Mix, Promega, Madison, USA) in 25 μl reaction mixtures containing 2 μl of each primer, 12.5 μ1 of master mix, 7.5 μl of distilled water, and 1 μl of genomic DNA template. Polymerase chain reaction (PCR) was performed using a thermal cycler (C1000 Touch^{TM} Thermal cycler, BioRad, USA) according to the following procedure: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 45s and extension at 72°C for 1 min 30s, and followed by final extension at 72°C for 10 min. The PCR products were subsequently visualized by electrophoresis on a 1.2% agarose gel. A single band was observed and then purified using the gel doc (U:Genius 3, Syngene, Frederick, USA) purification kit (NucleoSpin Gel and PCR Clean-up, Macherey-Nagel, Germany).

Results

Distribution of rice weevil, Sitophilus oryzae, in South Korea

From 2015 to 2016, we surveyed 52 RPCs located throughout South Korea using corrugated cardboard traps (30 × 30 cm) and by sampling rice grains. Populations of *S. zeamais* were collected from all RPCs surveyed, but a total of four populations of *S. oryzae* were collected from Cheongju and Boeun at Chungcheongbuk-do, Gunsan at Jeollabuk-do and Jangseong at Jeollanam-do (Figure 1). These data are the first to confirm *S. oryzae* populations within South Korea.

Morphological characters for identifying members of the Sitophilus weevil group

This study confirmed that *S. zeamais* and *S. oryzae* are distributed in Korea. *S. zeamais* is a major pest of stored grain, especially maize and milled rice, in the tropics and subtropics (Booth et al 1990; CABI 2015). This organism is very similar to *S. oryzae,* and the two species may occur during the same infestation (Booth et al 1990; CABI 2015). However, *S. zeamais* is usually blacker, has fine microsculpture, is more shiny, and is generally slightly larger than *S. oryzae* (Booth et al 1990). The antennal funicle II of *S. zeamais* is also longer than antennal funicle I (Egorov and Zherikhin 1996). Additionally, the scutellum has lateral elevations further apart than their longitudinal length, which is about half as long as the scutellum (Yoshida et al 1989). However, these external characters are not entirely reliable and dissection is usually required for specific identification (Figures 2 and 3; Booth et al 1990).

In this study, we found that these two species could be accurately identified by observation of adults for specific characteristics (Figure 4). Specifically, the median lobe of the aedeagus has two longitudinal grooves dorsally, except in the apical quarter; thus, the

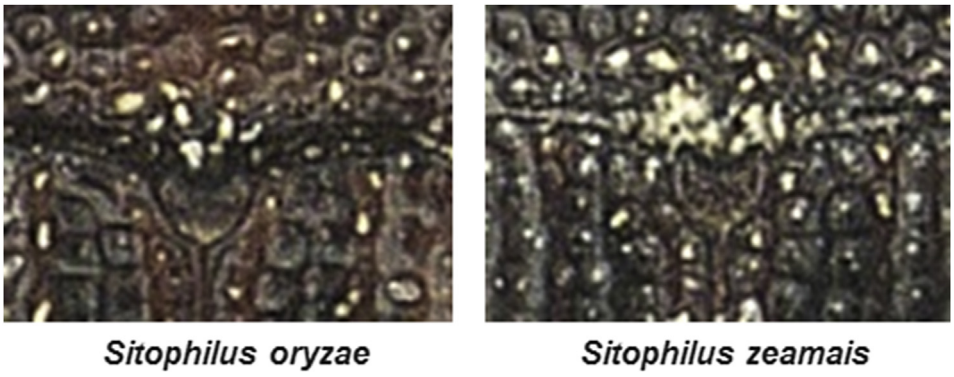

Figure 3. Comparisons of lateral elevation of scutellum for identification of *Sitophilus zeamais* and *S. oryzae* described by Yoshida et al (1989). However, this characteristic is not entirely reliable for their identification.

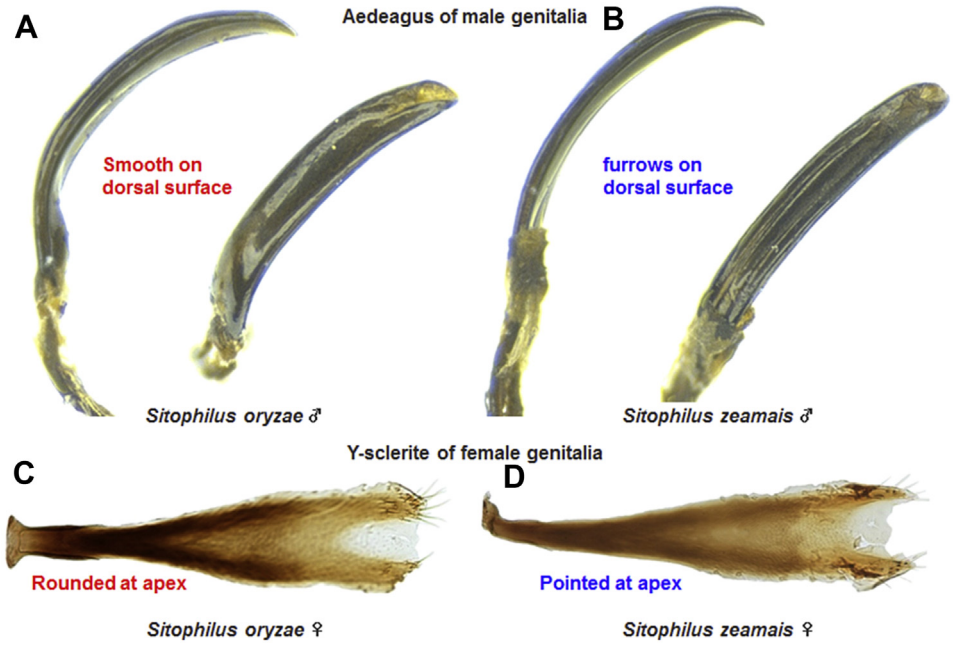

Figure 4. Aedeagus of male genitalia: A, *Sitophilus oryzae*; B, *Sitophilus zeamais*. Y-sclerite of female genitalia: C, *Sitophilus oryzae*; D, *Sitophilus zeamais*.

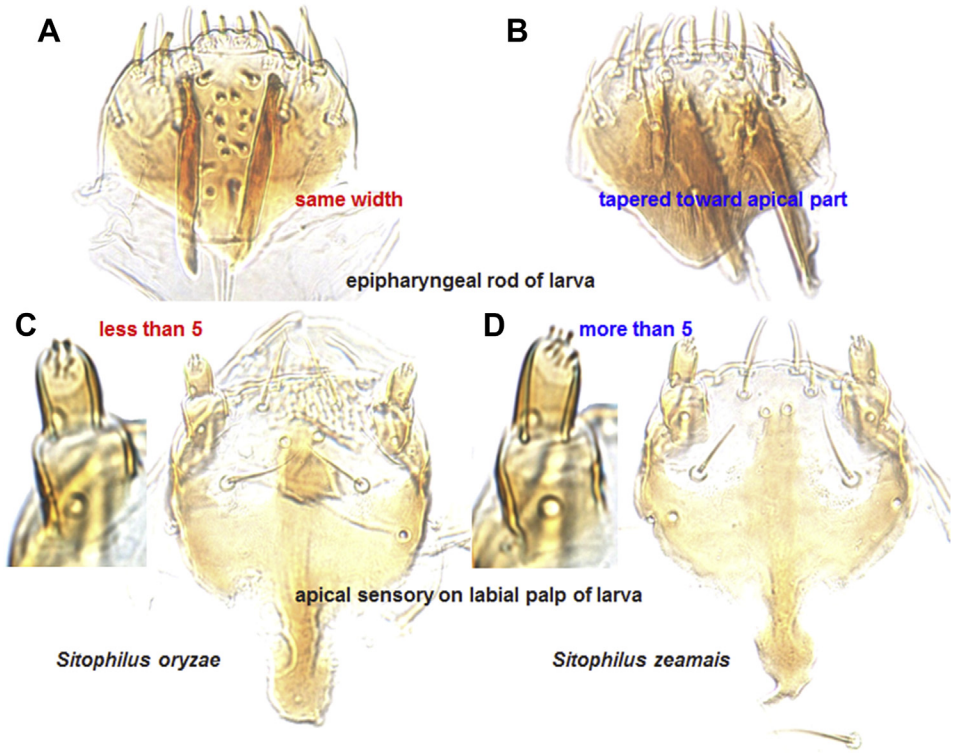

Figure 5. Epipharyngeal rod of larva. A, *Sitophilus oryzae*; B, *Sitophilus zeamais*. Apical sensory organs on the labial palp of larva: C, *Sitophilus oryzae*; D, *Sitophilus zeamais*.

male genitalia of *S. zeamais* are sinuous in cross-section. However, there is no longitudinal groove dorsally; therefore, the male genitalia are smoothly convex in cross-section for *S. oryzae* (Booth et al 1990; Zimmerman 1993). The lateral lobes of the Y-shaped sclerite are pointed at the apex, and their separation is greater in female genitalia of *S. zeamais*, but they are rounded at the apex and narrower in female genitalia of *S. oryzae* (Booth et al 1990). Moreover, in larva (Figure 5), the epipharyngeal rod is tapered toward the apical portion, and there are more than five apical sensory organs on the labial palp in *S. zeamais*, whereas the width and overall length are the same, but there are less than five apical sensory organs in *S. oryzae* (Gorham 1987).

Globally, *Sitophilus* also contains two other major economic pests. *S. granarius* is primarily a pest of wheat and barley stored under temperate conditions; while in tropical regions, it is limited to cooler, upland areas. *S. granarius* has rather elongate punctures

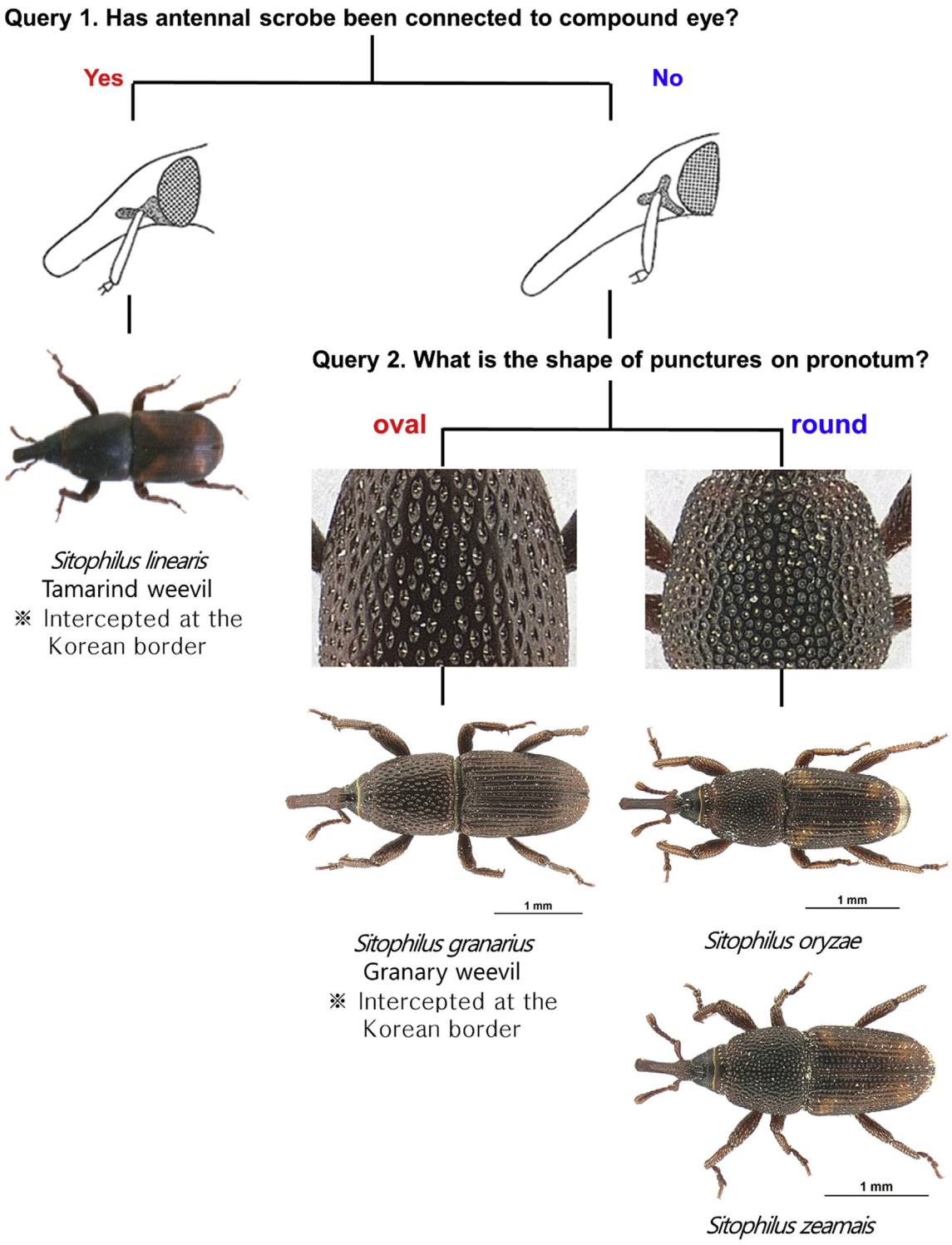

Figure 6. Pictorial identification key for *Sitophilus* weevil group thorough matching pictures of specific characteristic corresponding to the query.

on the pronotum (CABI 2015). The other pest in this group is *S. linearis*, which is capable of causing economic damage to the seeds of tamarind (Cotton 1920). These two species were intercepted at the Korean borders by the Animal and Plant Quarantine Inspection Agency (QIA), Korea (data from the database of the Pest Information System operated by the QIA).

A pictorial identification key (Figure 6) was generated to easily identify major pests of the *Sitophilus* weevil group by matching pictures of specific characteristics corresponding to the query. This key is composed of four queries for two Korean species and two exotic species and can be used by warehouse managers, entomological researchers, and quarantine inspectors.

Distinguished Sitophilus oryzae from S. zeamais using specific primers

We confirmed that PCR amplifications of the COI fragment were highly species-specific. Indeed, identification by the morphological characteristics corresponded with molecular identification for all

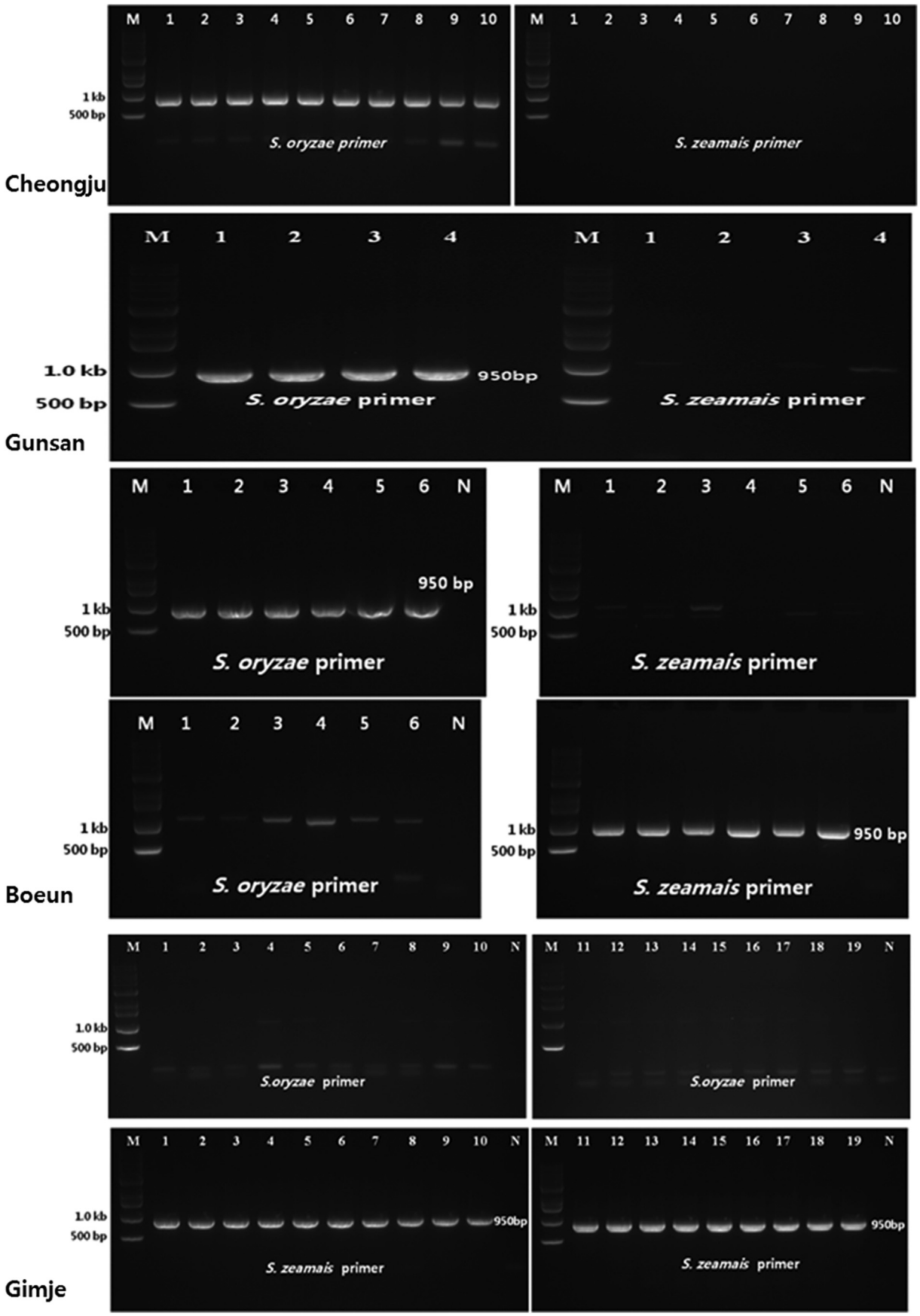

Figure 7. Distinguishing various populations of *Sitophilus oryzae* (Cheongju, Boeun and Gunsan) and *S. zeamais* (Boeun and Gimje) by PCR using specific primers.

30 samples of the three populations of *S. oryzae* and two populations of *S. zeamais* collected from Cheongju and Boeun (population mixed two species) at Chungcheongbuk-do and Gunsan and Gimje at Jeollabuk-do. For *S. zeamais* and *S. oryzae*, amplification yielded a single band with a size of about 950 bp (Figure 7). In Brazil, this molecular strategy was employed with male genitalia inspection to survey the distribution of both maize and rice weevils (Corrêa et al 2013).

Discussion

Sitophilus weevils, which are often detected found among grains stored in grain elevators, induce a large amount of economic damage. Currently, fumigants are known to be the most effective method to manage these insects. In Korea, phosphine has commonly been used to control insect pests of stored grains. To date, resistance to phosphine fumigant has not been reported for *S. zeamais*; however, it has been reported for *S. oryzae* (Nguyen et al 2015). In this study, we identified *S. oryzae* in Korea for the first time, suggesting that development of resistance to phosphine by *S. oryzae* collected in Korea should be checked in Korea.

Because *S. oryzae* populations that are resistant to phosphine can be introduced when importing grain from foreign countries, means for their control should be developed. In addition, it is necessary to ensure close quarantine inspections for undistributed *Sitophilus* weevils such as *S. granarius* and *S. linearis*.

Conflicts of interest

The authors declare that there is no conflicts of interest.

Acknowledgments

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